Development of Fibrinogen-based Bioinks for 3D Bioprinting Motor Neuron Progenitor Cells

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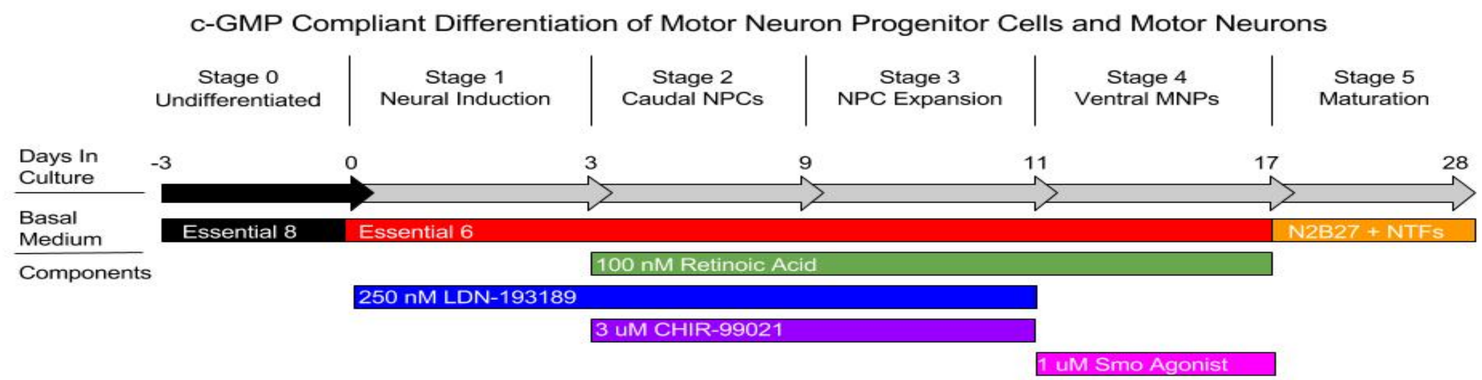
Introduction

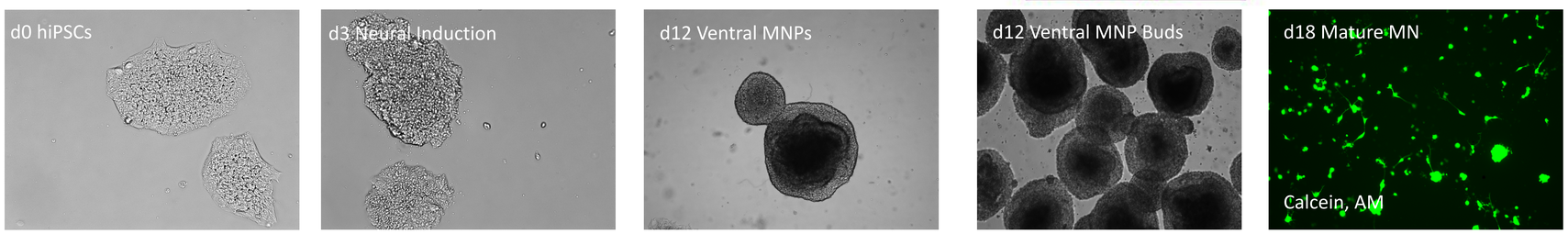
After a spinal cord injury, a lesion forms at the site of injury which inhibits the regeneration of axons. Drs. Ann Parr and Michael McAlpine currently have a collaborative grant to develop a 3D bioprinted, customizable spinal cord scaffold loaded with motor neuron progenitor cells (MNPCs) that can be inserted in this lesion as a potential treatment for spinal cord injury. Previously, the lab has used gelatin methacrylate (GELMA) and Matrigel, a murine Engelbreth-Holm-Swarm (EHS) sarcoma extract as potential bioinks. Gelma is believed to produce free radicals during the photo-polymerization step of bioprinting, resulting in low cell viability. Matrigel is composed of poorly controlled mixtures of growth factors and basement membrane proteins. Batch to batch variation of Matrigel leads to inconsistent 3D bioprinting settings and thus, a fully defined bioink that results in high viability of MNPCs must be developed.

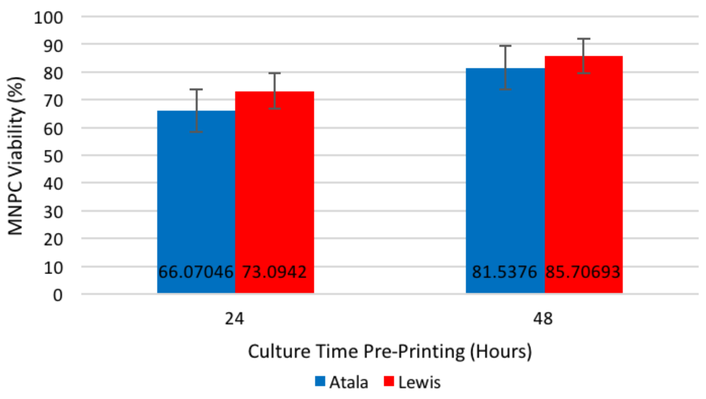
Methods

Prior to the production of fibrinogen-based bioink, a bank of MNPCs derived from human induced pluripotent stem cells (hIPSC) was produced using a protocol developed in the Parr lab. Different variations of fibrinogen-based bioinks were then prepared based on previous work by Lewis et al. and Atala et al. The Primary components of these bioinks include fibrinogen, gelatin, glycerol, and hyaluronic acid. MNPCs were them suspended into each bioink and plated. The bioink which resulted in highest overall cell viability was sent to printing. The cell-laden bioink was then printed into 3D scaffolds. Cell viability was again assessed to determine cell viability following the 3D bioprinting process. Using immunohistochemistry, differentiation of MNPCs was assessed after the addition of multiple growth factors. The final fibrinogen bioink composition consisted of 10 mg/mL fibrinogen, 7.5 wt/vol% gelatin, 2.5 mM $CaCl_2$, and 3 mg/mL HA. These components were diluted from stock solution using either DMEM basal media or N2B27 growth media.

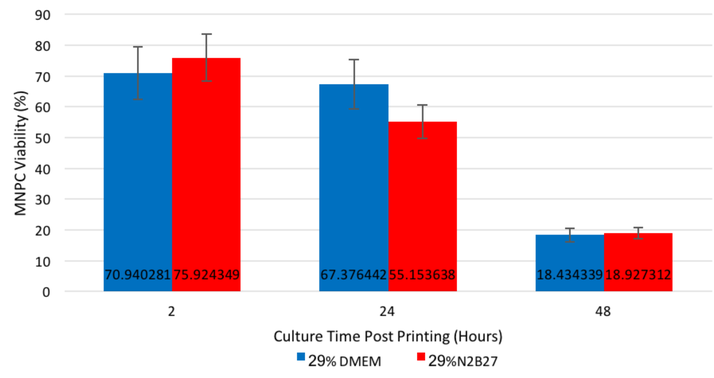
Results







MNPC 2D culture viability pre-printing process.



MNPC 2D culture viability post-printing process in modified fibrinogen-based bioinks.

Conclusions

In conclusion, a fibrinogen-based bioink was developed based on previous literature that is capable of promoting viability and differentiation of MNPCs when plated before the printing process. However, when plating MNPCs after the printing process, viability and differentiation are greatly reduced, indicating that the printing process itself is adversely affecting the viability and differentiation of these cells and that this process needs optimization before moving forward.

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